

Applicant : Gunnar Norstedt et al.
Serial No. : 08/963,288
Filed : November 3, 1997
Page : 4 of 13

Attorney's Docket No.: 13425-128001 / PH-1521-US

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. (Previously Presented) An in vitro method of enhancing the transcription of a gene in a DNA construct when the DNA construct is incorporated into the genome of a eukaryotic host cell, the method comprising:

(a) providing a DNA construct comprising a structural gene for a desired protein or polypeptide, a gene promoter upstream of and operably linked to the structural gene, and six copies of an enhancer element upstream of the promoter;

(b) transfecting the eukaryotic host cell to incorporate the DNA construct into the genome of the host cell; and

(c) exposing the DNA construct in the eukaryotic host cell to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof;

wherein the enhancer element comprises the nucleotide sequence TTCTGAGAA, with the proviso that the nucleotide sequence does not contain the DNA sequence of nucleotide sequence SEQ ID NO:1, and wherein the enhancer element is responsive to both lactogenic hormones and somatogenic hormones.

2. (Previously Presented) The method according to claim 1, wherein the enhancer element consists essentially of the nucleotide sequence TTCTGAGAA.

3-7. (Cancelled)

Applicant : Gunnar Norstedt et al.
Serial No. : 08/963,288
Filed : November 3, 1997
Page : 5 of 13

Attorney's Docket No.: 13425-128001 / PH-1521-US

8. (Previously Presented) An expression vector comprising a structural gene encoding a desired protein or polypeptide and a promoter, wherein the vector further comprises six enhancer elements, and further wherein each of the enhancer elements consists essentially of the nucleotide sequence SEQ ID NO:1.

9. (Previously Presented) An expression vector according to claim 8, wherein the promoter is a thiamine kinase promoter.

10. (Previously Presented) An expression vector comprising a structural gene encoding a desired protein or polypeptide and a promoter, wherein the vector further comprises six enhancer elements, and further wherein each of the enhancer elements consists essentially of the nucleotide sequence TTCTGAGAA.

11. (Previously Presented) An isolated eukaryotic host cell containing the expression vector according to claim 8.

12-15. (Cancelled)

16. (Previously Presented) An isolated eukaryotic host cell containing the expression vector according to claim 9.

17. (Previously Presented) An isolated eukaryotic host cell containing the expression vector according to claim 10.

18. (Cancelled)

19. (Previously Presented) An in vitro method of enhancing the transcription of a gene in a DNA construct, the method comprising:

Applicant : Gunnar Norstedt et al.
Serial No. : 08/963,288
Filed : November 3, 1997
Page : 6 of 13

Attorney's Docket No.: 13425-128001 / PH-1521-US

- (a) providing a first DNA construct comprising a structural gene and a promoter upstream of the structural gene,
- (b) incorporating the nucleotide sequence consisting of TTCTGAGAA into the first DNA construct upstream of the promoter, thereby producing a second DNA construct,
- (c) transfecting a eukaryotic host cell to incorporate the second DNA construct into the genome of the host cell; and
- (d) exposing the second DNA construct in the eukaryotic host cell to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof.

20. (Previously Presented) A method according to claim 19, wherein the hormone is selected from the group consisting of growth hormone, prolactin, placenta lactogen and mixtures thereof.

21. (Previously Presented) A method according to claim 20, wherein the hormone is selected from the group consisting of prolactin, placenta lactogen and mixtures thereof.

22-33. (Cancelled)

34. (Previously Presented) An in vitro method of enhancing the transcription of a gene in a DNA construct comprising a structural gene, a promoter upstream of the structural gene, and at least one enhancer upstream of the promoter; the method comprising
placing the DNA construct in an environment wherein transcription can occur; and
exposing the DNA construct to a hormone selected from the group consisting of lactogenic hormones, somatogenic hormones and mixtures thereof;
wherein the enhancer element consists essentially of the nucleotide sequence
TTCTGAGAA.

35. (Previously Presented) A method according to claim 34, wherein the hormone is

Applicant : Gunnar Norstedt et al.
Serial No. : 08/963,288
Filed : November 3, 1997
Page : 7 of 13

Attorney's Docket No.: 13425-128001 / PH-1521-US

selected from the group consisting of growth hormone, prolactin, placenta lactogen and mixtures thereof.

36. (Previously Presented) A method according to claim 35, wherein the hormone is selected from the group consisting of prolactin, placenta lactogen and mixtures thereof.

37-38. (Cancelled)

39. (Previously Presented) A method according to claim 1, wherein the hormone is selected from the group consisting of growth hormone, prolactin, placenta lactogen and mixtures thereof.

40. (Previously Presented) A method according to claim 39, wherein the hormone is prolactin.

41. (Previously Presented) An in vitro method of enhancing transcription of a structural gene, comprising the steps of:

- (a) preparing a plasmid DNA construct comprising a structural gene, a promoter upstream of the structural gene, and at least one enhancer consisting of the sequence TTCTGAGAA upstream of the promoter;
- (b) transfecting a cell with the plasmid DNA construct; and
- (c) exposing the cell to prolactin.

42. (Previously Presented) An in vitro method according to claim 41, wherein the plasmid DNA comprises up to six enhancers.

43. (Cancelled)

Applicant : Gunnar Norstedt et al.
Serial No. : 08/963,288
Filed : November 3, 1997
Page : 8 of 13

Attorney's Docket No.: 13425-128001 / PH-1521-US

44. (Previously Presented) An isolated DNA construct comprising a promoter operably linked to a structural gene downstream from said promoter, and six repeats of an enhancer element upstream from said promoter, wherein the enhancer element consists essentially of the sequence TTCTGAGAA.

45. (Previously Presented) An isolated DNA construct according to claim 44, wherein the enhancer consists of the sequence TTCTGAGAA.

46. (Previously Presented) An in vitro method of enhancing the transcription of a gene, the method comprising the steps of:

- (a) providing a DNA construct comprising the gene, a promoter upstream of the gene, and at least one copy of the nucleotide sequence TTCTGAGAA upstream of the promoter;
- (b) transfecting the cell with the DNA construct, and
- (c) exposing the DNA construct to prolactin.

47. (Previously Presented) An in vitro method according to claim 46, wherein the DNA comprises multiple copies of the nucleotide sequence TTCTGAGAA.

48. (Previously Presented) An in vitro method according to claim 47, wherein the DNA construct comprises six copies of the nucleotide sequence TTCTGAGAA.

49. (Cancelled)

50. (Previously Presented) An in vitro method according to claim 34, wherein the transfecting step comprises transfecting the eukaryotic cell with a plasmid comprising the DNA construct.

51. (Cancelled)

Applicant : Gunnar Norstedt et al.
Serial No. : 08/963,288
Filed : November 3, 1997
Page : 9 of 13

Attorney's Docket No.: 13425-128001 / PH-1521-US

52. (Previously Presented) The method according to claim 2, wherein the enhancer element consists of the nucleotide sequence TTCTGAGAA.

53-54. (Cancelled)

55. (Previously Presented) An in vitro method of enhancing transcription of a nucleotide sequence, the method comprising:

providing a eukaryotic cell comprising an expression vector comprising (i) a nucleotide sequence encoding a desired protein, (ii) a promoter upstream of the nucleotide sequence, and (iii) an element upstream of the promoter comprising the sequence TTCTGAGAA; and

contacting the cell with a lactogenic hormone, thereby enhancing transcription of the nucleotide sequence.

56. (Currently Amended) The method of claim 55 54, wherein the lactogenic hormone is prolactin.

57. (Currently Amended) The method of claim 55 54, wherein the element comprises the sequence

GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCATG (SEQ ID NO:1).

58. (Currently Amended) The method of claim 55 54, wherein the element comprises six copies of the sequence TTCTGAGAA.

59. (Currently Amended) The method of claim 55 54, wherein the element comprises six copies of the sequence

GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCATG (SEQ ID

Applicant : Gunnar Norstedt et al.
Serial No. : 08/963,288
Filed : November 3, 1997
Page : 10 of 13

Attorney's Docket No.: 13425-128001 / PH-1521-US

NO:1).

60. (Previously Presented) An in vitro method of enhancing transcription of a nucleotide sequence, the method comprising:

providing an expression vector comprising (i) a nucleotide sequence encoding a desired protein, (ii) a promoter upstream of the nucleotide sequence, and (iii) an element upstream of the promoter comprising the sequence TTCTGAGAA;

transfected a eukaryotic cell with the expression vector; and

contacting the cell with a lactogenic hormone, thereby enhancing transcription of the nucleotide sequence.

61. (Currently Amended) The method of claim 60 59, wherein the lactogenic hormone is prolactin.

62. (Currently Amended) The method of claim 60 59, wherein the element comprises the sequence

GATCTACGCTTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCATG (SEQ ID NO:1).

63. (Currently Amended) The method of claim 60 59, wherein the element comprises six copies of the sequence TTCTGAGAA.

64. (Currently Amended) The method of claim 60 59, wherein the element comprises six copies of the sequence

GATCTACGCTTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCATG (SEQ ID NO:1).

65. (Previously Presented) An in vitro method of enhancing transcription of a

Applicant : Gunnar Norstedt et al.
Serial No. : 08/963,288
Filed : November 3, 1997
Page : 11 of 13

Attorney's Docket No.: 13425-128001 / PH-1521-US

nucleotide sequence, the method comprising:

constructing an expression vector comprising (i) a nucleotide sequence encoding a desired protein, (ii) a promoter upstream of the nucleotide sequence, and (iii) an element upstream of the promoter comprising the sequence TTCTGAGAA

transfected a eukaryotic cell with the expression vector; and

contacting the cell with a lactogenic hormone, thereby enhancing transcription of the nucleotide sequence.

66. (Currently Amended) The method of claim 65 64, wherein the lactogenic hormone is prolactin.

67. (Currently Amended) The method of claim 65 64, wherein the element comprises the sequence
GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCATG (SEQ ID NO:1).

68. (Currently Amended) The method of claim 65 64, wherein the element comprises six copies of the sequence TTCTGAGAA.

69. (Currently Amended) The method of claim 65 64, wherein the element comprises six copies of the sequence
GATCTACGCTTCTACTAATCCATGTTCTGAGAAATCATCCAGTCTGCCATG (SEQ ID NO:1).